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Generation of nanobody dimers by sortase-mediated functionalization and click chemistry

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An abbreviated version of this protocol was published in Science Advances in Mar 2022

Multivariate mining of an alpaca immune repertoire identifies potent cross-neutralizing SARS-CoV-2 nanobodies

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Detailed protocol

To generate nanobody dimers using sortase-mediated functionalization and click chemistry, one nanobody is functionalized with a DBCO and the other with an azide. This not only facilitates the creation of homodimers, but also heterodimers. A prerequisite for this method is that the nanobodies are expressed with a C-terminal sortase recognition motif. For our experiments, the nanobodies are expressed with the following C-terminal sequence: GGLPETGGHHHHHH.

It is worth noting that the specific reaction conditions may require adjustment depending on the protein used. Variations in reaction efficiency can occur even between different nanobodies, hence it is essential to monitor the labeling process carefully and modify the conditions as needed for optimal yield. Throughout the reaction steps, we recommend taking aliquots to monitor the progress and success of the reactions using SDS-PAGE or LC-MS.

The nanobodies, at concentrations ranging from 75-100 µM, are incubated with 5 µM sortase A and either 8 mM DBCO-amine (Sigma-Aldrich, #761540) or 10 mM 3-azido-1-propanamine (Sigma-Aldrich, #762016). This is performed in 50 mM tris buffer (pH 7.5), 150 mM NaCl, and 10 mM $CaCl_2$. The reaction is allowed to proceed for 3 hours at 25 °C, but both the time and temperature can be adjusted, if required, to improve yield.

Following this, unreacted nanobody, sortase A, and excess nucleophile are removed using Ni-NTA resin and desalting columns (Seba spin or PD-10 desalting columns).

To generate the dimers, equal molar amounts of a DBCO and an azide-labeled nanobody are mixed together. The mixture is incubated at 4°C until most nanobodies are dimerized (24-72h). Dimers are separated from unreacted monomers using size-exclusion chromatography.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Hanke, L. (2023). Generation of nanobody dimers by sortase-mediated functionalization and click chemistry. Bio-protocol Preprint. bio-protocol.org/prep2309.
2. Hanke, L., Sheward, D. J., Pankow, A., Vidakovics, L. P., Karl, V., Kim, C., Urgard, E., Smith, N. L., Astorga-Wells, J., Ekström, S., Coquet, J. M., McInerney, G. M. and Murrell, B.(2022). Multivariate mining of an alpaca immune repertoire identifies potent cross-neutralizing SARS-CoV-2 nanobodies. Science Advances 8(12). DOI: 10.1126/sciadv.abm0220